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60/156,677 29 September 1999 (29.09.1999) US
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- (74) Agents: **REED, T., David et al.**; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217-1087 (US).
- (81) Designated States (national): AE, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: METHODS AND KITS FOR SEQUENCING POLYPEPTIDES

(57) Abstract: The present disclosure provides methods and kits which are useful for sequencing polypeptides. The methods involve derivatization of the N-terminus of the polypeptide or peptides thereof. Mass spectral analysis of one or more of the resulting derivatized analytes provides spectra which are readily interpreted through the use of techniques well-known to the ordinarily skilled artisan. The present disclosure also describes kits which enhance convenient performance of the methods.

**PATENT COOPERATION TREATY**  
**PCT**

**INTERNATIONAL SEARCH REPORT**

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference  7379M/MH	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No.  PCT/US 00/ 00790	International filing date ( <i>day/month/year</i> )  12/01/2000	(Earliest) Priority Date ( <i>day/month/year</i> )  20/01/1999
Applicant  THE PROCTER & GAMBLE COMPANY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
  - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
  - contained in the international application in written form.
  - filed together with the international application in computer readable form.
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  - furnished subsequently to this Authority in computer readable form.
  - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
  - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.  **Certain claims were found unsearchable** (See Box I).

3.  **Unity of Invention is lacking** (see Box II).

4. With regard to the **title**,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. \_\_\_\_\_

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00790

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BEILSTEIN Data, EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>COX K A ET AL: "Role of the Site of Protonation in the Low-Energy Decompositions of Gas-Phase Peptide Ions" JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, US, ELSEVIER SCIENCE INC, vol. 7, no. 6, 1 June 1996 (1996-06-01), pages 522-531, XP004052038 ISSN: 1044-0305 cited in the application page 523, column 2, line 13, paragraph 2 - line 24</p> <p>---</p> <p>-/-</p>	1-10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

**° Special categories of cited documents :**

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

3 July 2000

17/07/2000

Name and mailing address of the ISA

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Hart-Davis, J

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00790

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NAKANISHI T ET AL: "Electrospray Ionization--Tandem Mass Spectrometry Analysis of Peptides Derived by Enzymatic Digestion of Oxidized Globin Subunits: An Improved Method to Determine Amino Acid Substitution in the Hemoglobin 'Core'" JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, US, ELSEVIER SCIENCE INC, vol. 7, no. 10, 1 October 1996 (1996-10-01), pages 1040-1049, XP004070144 ISSN: 1044-0305 the whole document ---	1-10
A	ROTH K.D.W., HUANG Z.-H., SADAGOPAN N., WATSON J.T.: "Charge derivatization of peptides for analysis by mass spectrometry" MASS SPECTROM. REV., vol. 17, 1998, page 255 XP000922702 the whole document ---	1-10
A	HUANG Z.-H., WU J., ROTH K.D.W., YANG Y., GAGE D.A., WATSON J.T.: "A picomole-scale method for charge derivatization of peptides for sequence analysis by mass spectrometry" ANALYTICAL CHEMISTRY, vol. 69, 1997, pages 137-144, XP002141708 the whole document ---	1-10
P, X	KEOUGH, T.; YOUNGQUIST, R. S.; LACEY, M. P.: "A method for high-sensitivity peptide sequencing using postsource decay matrix-assisted laser desorption ionization mass spectrometry" PROC. NATL. ACAD. SCI. U. S. A., vol. 96, June 1999 (1999-06), pages 7131-7136, XP002141709 the whole document ---	1-10
T	DATABASE CHEMICAL ABSTRACTS 'Online! American Chemical Society (via STN, Karlsruhe); Accession Number 2000:391605 (CAPLUS), BAUER, MARK D.; SUN, YIPING; KEOUGH, THOMAS ; LACEY, MARTIN P.: "Sequencing of sulfonic acid derivatized peptides by electrospray mass spectrometry" XP002141711 abstract & RAPID COMMUN. MASS SPECTROM. , vol. 14, no. 10, 2000, pages 924-929, ---	1-10
	-/-	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00790

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	HUANG ZHI-HENG; SHEN TUNLI; WU JIANG; GAGE DOUGLAS A; WATSON J THROCK: "Protein sequencing by matrix-assisted laser desorption ionization-postsource decay-mass spectrometry analysis of the N-Tris(2,4,6-trimethoxyphenyl)phosphine-acetylated tryptic digests" ANALYTICAL BIOCHEMISTRY, vol. 268, 15 March 1999 (1999-03-15), pages 305-317, XP002141710 the whole document ---	1-10
P,A	CHEN X ET AL: "Isotope Edited Product Ion Assignment by alpha-N Labeling of Peptides with 'H3(50%)!2,4-Dinitrofluorobenzene" JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, US, ELSEVIER SCIENCE INC., NEW YORK, NY, vol. 10, no. 5, May 1999 (1999-05), pages 448-452, XP004164485 ISSN: 1044-0305 the whole document -----	1-10